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"Formulation and Evaluation of Topical Delivery System for the Treatment of *Acne Vulgaris*"

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ABSTRACT

Selected drug *Cynodon dactylon* slightly hydrophobic in nature which limits therapeutic concentration of drug at target site. *C. dactylon* contain ferulic acid as an active constituent confirmed by HPTLC analysis.MIC value of *C. dactylon* against *P. acne* was 0.624 mg/ml. Chitosan nanoparticles are now thought to be most effective formulation; biocompatible, biodegradable, less harmful and easy to use in preparation. The objective of this study was to synthesis chitosan vanillin cross-linked nanoparticles and to formulate herbal topical nanogel to treat acne. The nanoparticles prepared by emulsion solvent evaporation method & characterized. The optimized batch of *C. dactylon* nanoparticles (F6) had particle size 320nm, PDI 0.5, Zeta potential (-22.7mV) & %EE 72.23±0.5 %. It was observed that as polymer concentration increases particle size & % EE also increases. These optimized nanoparticles transformed into topical nanogel. The gel formulation with 1% carbopol showed good pH, viscosity, homogeneity, spredability, % drug content (90.32±0.31 %) &% drug release (74.19±0.2 %), zero irritancy score & good antibacterial activity against *P. acne.* Hence, chitosan nanoparticles of *C. dactylon* in aqueous gel base can be used as an appropriate formulation to treat *acne vulgaris*.

Keywords: *Cynodon dactylon*, Nanoparticles, Chitosan, Vanillin, Nanogel.

INTRODUCTION

Acne is a common chronic skin disease occurs when hair follicle plugs with oil and dead skin cells, marked by blackheads, pustules and nodules. Acne can be caused by variety of causes including heredity, dietary habits, psychological issues, the activity of the sebaceous glands and bacterial infections. *Propionibacterium acnes* is one of the most common bacteria that causes acne. *P. acnes* is a normal flora of the human's skin's pilosebaceous glands and it causes acne by producing lipase, which breakdown free fatty acid from skin lipids and cause tissue inflammation. During repeated therapy, specific association between antibiotic and bacteria forms resistance to antibiotics. Because of negative side effects associated with synthetic medications, the use of herbal therapy has increased.

One of such plant considered of great importance is *Cynodon dactylon* a creeping grass belonging to family Poaceae.Phenolic phytotoxins (Ferulic, Syringic, P-coumaric, Vanillic) the active principles of *Cynodon dactylon*).It has the reported potent antibacterial, anti-inflammatory & antioxidant activity. But, due to its low aqueous solubility, preparation of topical dosage form is critical task for the formulator. Among the various polymeric nanoparticles, chitosan nanoparticles are thought to be effective against bacteria and viruses.

As they are biocompatible, biodegradable, less toxic and easy to use in preparation. Vanillin is used as natural cross-linker to form stable nanoparticles. It is most commonly used as flavoring agent in food, drink and cosmetic. Some articles are present on cross-linking of chitosan using vanillin. Over other topical formulations (oils, lotions, creams) nanogels have advantages like high stability, high drug loading capacity, can hydrate skin without clogging pores& controlled drug release[1,2].

MATERIALS AND METHODS

Materials:

The plant material *Cynodon dactylon (*L) was collected from Hadapsar, Maharashtra and authenticated (AUTH 22-35).from Agharkar Research institute, Pune. All other chemicals agents used were of analytical grade.

Methods:

Preparation of extract

Cynodon dactylon (L.) Pers. Collected & allowed to drying and then in a grinder it is crushed to powdered form. The powder is passed through 120 meshes to separate fine powder from the coarse powder. The coarse powdered drug so obtained is then employed for the method of extraction. Ethanoic solvent is used for the process of extraction by Soxhlet apparatus.

Preliminary phytochemical screening

Standard methods are employed to carry out phytochemical screening. Presences of substances like carbohydrates, glycosides, flavonoids, saponins, alkaloids, phenolic compounds, fixed oils, tannins, are found in the extract[6.]

HPTLC analysis study

To check purity of sample HPTLC analysis was done.Standard stock solution containing 1mg mL-1 of ferulic acid was prepared by dissolving 10 mg ferulic acid in 10 mL methanol. The stock solution was further diluted to attain final concentration of 25 g mL-1 for HPTLC analysis.Each of the concentrated extract was re-dissolved in methanol and filtered through 0.45m filter. Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 0.1, v/v/v) was used as mobile phase &HPTLC Silica MERK 60F 254 as stationary phase[7.]

Determination of MIC of *C. dactylon* ethanolic extract

For this, various concentration of *C. dactylon* extract (10mg/ml to 0.0195 mg/ml) were prepared in ethanol& MIC determined by broth dilution method.*C. dactylon* extract MIC value (0.624mg/ml) against *P. acne* indicates, extract can be used for the treatment of *Acne vulgaris*[5].

Preparation of Chitosan Vanillin nanoparticles

Chitosan vanillin nanoparticles were prepared by emulsion-solvent evaporation method. Chitosan was dissolved in aqueous acetic acid solution (10 ml, 1%, v/v) with a concentration of 2% (w/v) to form a water phase. Liquid paraffin (50 ml) containing 1% span 80 was used as oil phase. The chitosan solution was added drop wise into oil phase to form W/O emulsion by stirring for 1 hour at room temperature. Then, vanillin dissolved in acetone was dropped slowly into the emulsion under mechanical agitation at room temperature. The stirring was maintained till the entire evaporation of acetone. Chitosan nanoparticles were collected by centrifugation at 7500 rpm for 10 minutes at RT and washed with petroleum ether and isopropanol for three times. Nine batches were formulated by changing concentration of chitosan & vanillin[11,12].

Table 1: Composition of batches of *Cynodon dactylon* extract loaded Nanoparticles.

Formulation code	*C.dactylon* Extract	Chitosan	Vanillin	Liquid paraffin	Acetone	Tween 80
F1	0.2	0.25	0.2	5	5	1
F2	0.2	0.25	0.4	5	5	1
F3	0.2	0.25	0.6	5	5	1
F4	0.2	0.5	0.2	5	5	1
F5	0.2	0.5	0.4	5	5	1
F6	0.2	0.5	0.6	5	5	1
F7	0.2	0.75	0.2	5	5	1
F8	0.2	0.75	0.4	5	5	1
F9	0.2	0.75	0.6	5	5	1

Note: All values in %

Preparation of Gel

The gel was prepared by direct dispersion method by dispersing Carbopol 934 in water for 2 hr. for swelling. Once the carbopol 934 is been swelled it is kept on magnetic stirrer for stirring & then prepared nanoparticles added in carbopol mixture. To this, propylene glycol which acts as penetration enhancer is added into the mixture. All gel formulations were adjusted to pH 6, using triethanolamine & stirred slowly until clear gel was obtained. Three batches of gel formulated by varying conc. of Carbopol 934 i.e. 0.5%,1% & 1.5%[13].

Characterization of nanoparticles suspension

Prepared *C. dactylon* loaded chitosan nanoparticles suspensions were evaluated for physical examination, particle size, Zeta potential, PDI and entrapment efficiency.

Particle size, Zeta potential and Polydispersity index (PDI)

Dynamic Light Scattering (MAL1098084, Zetasizer 7.12, Malvern Instruments, UK) was used to measure the particle size and zeta potential (ZP) of all the *C. dactylon* loaded nanoparticles. All samples were diluted with DW for making suitable concentrations. The Z-average PS, PDI and zeta potential were determined.

Entrapment efficiency (%EE)

To determine EE (%) the concentration of unentrapped drug after separation was measured. Nanoparticles suspension was centrifuged at 12000 rpm for 30 min to separate the nanoparticles. Then the supernatant was diluted with ethanol & analyzed by UV spectroscopy.

Optimized nanoparticles batch was used for Scanning electron microscopy (SEM) and Fourier Transform Infra-Red Spectroscopy (FTIR) study.

Evaluation parameters of Gel

Physical characteristics& pH determination

The prepared nanogel formulations were inspected visually for their color, appearance and consistency.1 % aq. solution of gel formulation was prepared and stored for 2 h and pH was determined using a digital pH meter[16].

Viscosity & Spredability

The viscosity of the formulated batches decided by employing Brookfield viscometer with spindle no.64 at 10 rpm. Spreadability of gels was determined by Arvouet Grand Method by pressing 1 g of a sample between two 20 X 20 cm horizontal plates, the upper of which weighed 125 g. The spread diameter was measured after 1 min.

% Drug content

Gel was dissolved into 50 ml of ethanol & allowed to sonicate for 15 min to dissolve the *C. dactylon* completely into the ethanol. Filtered through whatman filter paper and resultant filtrate was diluted withy ethanol. Absorbance recorded at 414nm using UV spectrophotometer&% drug content calculated.[14]

% Drug Release

Release of *C. dactylon* (in vitro) from gel containing *C. dactylon* loaded nanoparticles (formulation 6) was estimated using Franz diffusion cell apparatus using Hen's egg membrane & pH 7.4 phosphate buffer. A sample was withdrawn at different time intervals of 0, 1, 2,3,4,5 hrs. &analyzed simultaneously by UV spectrophotometry at 414 nm.

Skin irritation test

Skin irritation study was carried out by HET-CAM (hen's egg chorioallantoic membrane) test. Using 0.9 % Nacl solution as negative control & 0.1 % SLS solution as positive control[15].

Antibacterial study

The microbial culture *Propionibacterium acne* (MTCC No. 1951) was used as the test strain, which was procured from the MTCC, Chandigarh. These inoculums were spread onto nutrient agar plate by streak method, which was from Eugreen Bio labs Kochi, Kerala. The reconstituted gel was tested for antibacterial activity using agar well diffusion method on MRCA media[9].

RESULTS AND DISCUSSIONS

The results of phytochemical screening of ethanolic extract of *C. dactylon* revealed presence of alkaloids, flavonoids, phenols, tannins etc. Confirmation of ferulic acid was done by matching the Rf values of drug sample and standard ferulic acid track & confirmed by UV-VIS spectra. Rf value of std. Ferulic acid was 0.43 &*C. dactylon*extract Rf value was 0.41 as shown in fig.no.2.

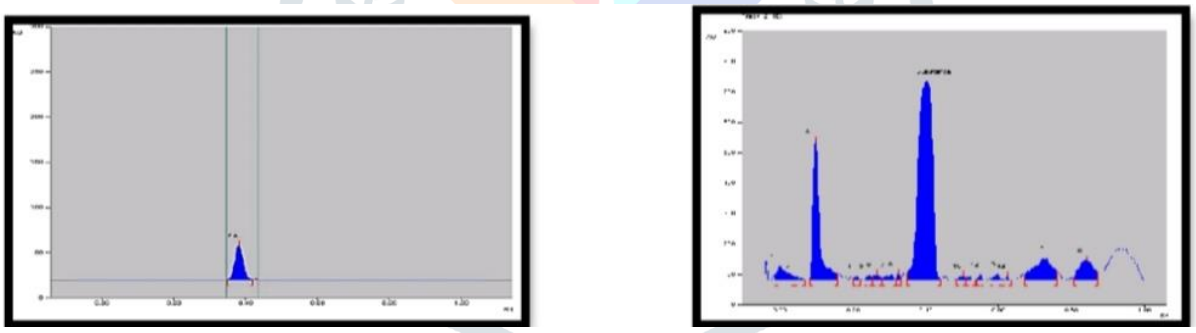

Fig. 1: HPTLC Chromatogram of standard Ferulic acid& Ethanolic extract of C. dactylon resp.

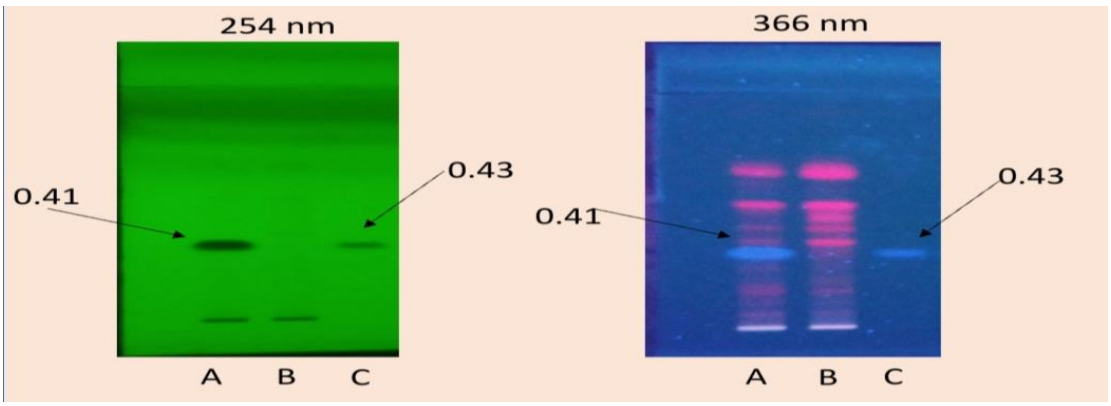

Fig.2: HPTLC fingerprinting of ethanolic extract of *C. dactylon* at 254 nm & 366 nm resp.

Where, A= 2µl *C. dactylon* extract, B= 3µl *C. dactylon* extract, C= 2µl standard Ferulic acid

C. dactylon loaded chitosan nanoparticles were formulated by solvent evaporation method. As a cross-linker vanillin was selected. Li et al (2014) stated that formation of chitosan nanoparticles is because of Schiff reaction between the amino group of chitosan & vanillin's aldehyde group, as well as formation of hydrogen bond between chitosan and vanillin. All nanoparticles formulations F1 to F9 were observed in light green color. % EE of all formulations was found to be in the range of 55.20±0.12 to 72.23±0.31 %. The zeta potential of nanoparticles indicates stability. Zeta potential value greater than +30 or less than -30 mV shows greater colloidal stability.PS & ZP was found to be in the range of 320±3.22 to 670±5.83 nm& -13.9±2.12 to -22.7± 1.01 mV resp. It was observed that as vanillin concentration increased, the magnitude of the zeta potential increased which could be due use of surfactant (Tween 80).

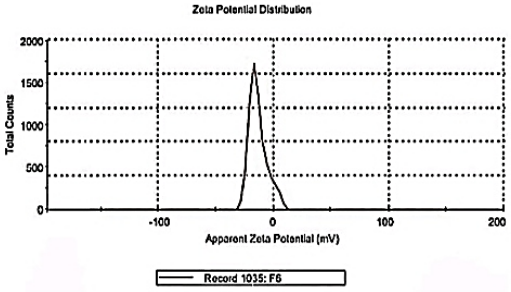

Fig.3: Particle size of F6 batch

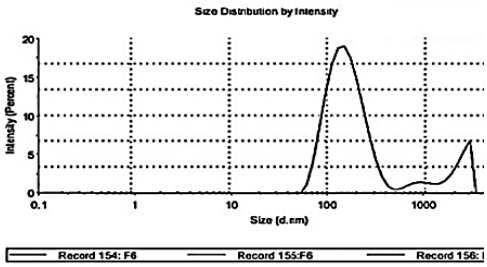

Fig.4: Zeta potential of F6 batch

In the spectrum of vanillin(fig.5C) the characteristic absorption peak at 3156 cm–1 was due to the vibration of -OH, the peak at 1666 cm^{-1} corresponds to stretching vibrations of C=O of aldehyde group, the peak at 1589,1510 and 812 cm^{-1}due to benzene ring of vanillin. In spectrum of chitosan (Fig.5B), the peak at 3300-3500 cm–1 is the stretching vibration of -OH and -NH, and the peaks at 2918 and 2877 cm–1 is due to the stretching vibration of C-H, the peak at 1382 cm^{-1}belongs to the stretching vibration of C-N bond. It is obvious from the FT-IR spectrum of *C. dactylon* loaded chitosan nanoparticles (Fig.5D) that the peak at 1644 $cm^{-1}$is due to the stretching vibration of C=N which confirms formation of nanoparticles due to interaction between amino group of chitosan and aldehyde group of vanillin.

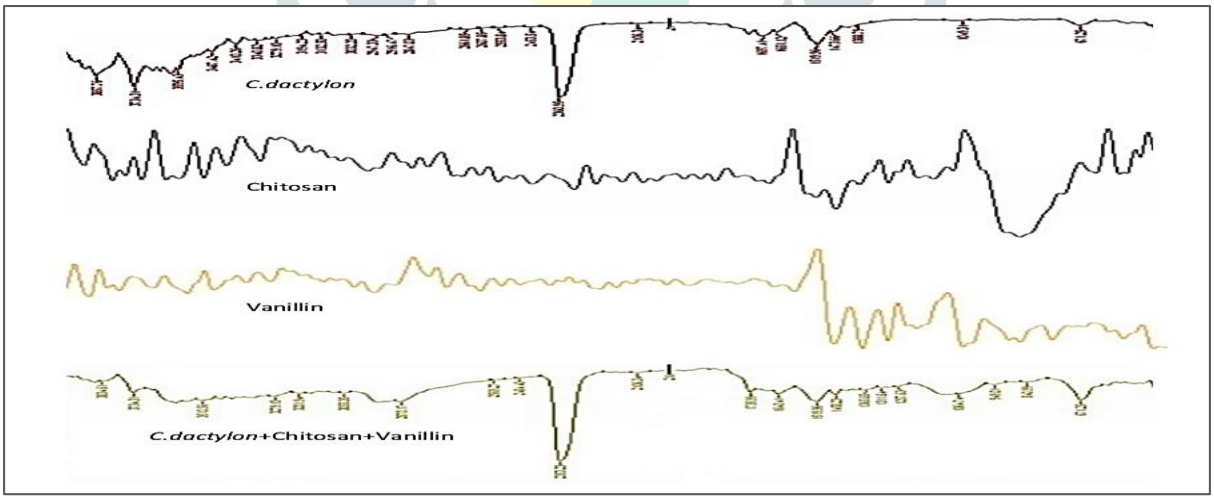

Fig.5: FTIR Spectrum of A) *C. dactylon* B) Chitosan C) Vanillin D) *C. dactylon* loaded chitosan nanoparticles.

All the characteristics peaks of *C. dactylon* were present in the spectrum of drug & physical mixture, indicating compatibility between drug+ polymer+ cross-linker. The spectrum confirmed that there is no significant change in absorption band, hence no interaction between them. However, some additional peaks were observed with physical mixtures, which could be due to presence of polymer.

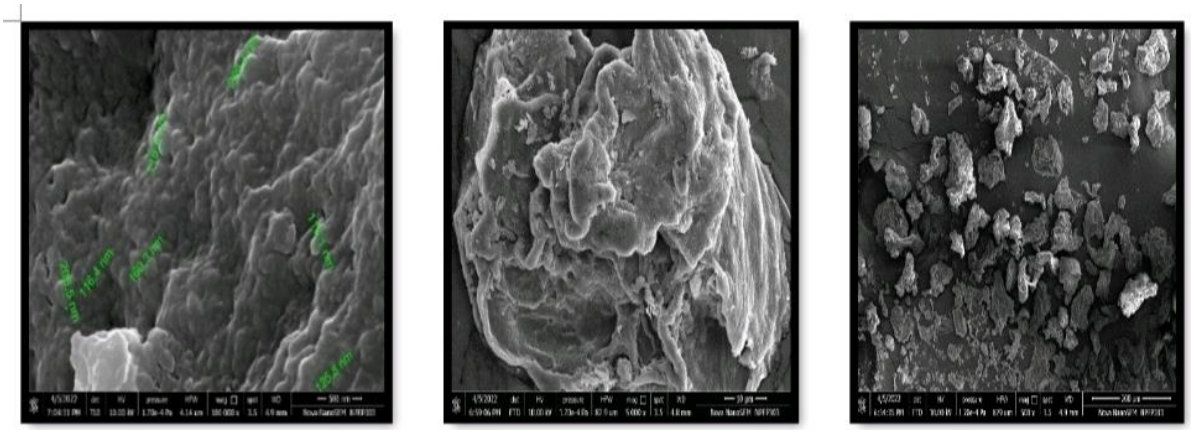

Fig.6: Scanning Electron Microscopy (SEM) of optimized nanoparticles.

SEM analysis of optimized nanoparticles showed agglomerated, nearly circular shaped nanoparticles with rough surface as shown in fig.6.

All the Carbopol 934 gel formulations were light green colored, semisolid with smooth consistency and homogeneity. It was washable. &pH was in the range of 6.1±0.5 to6.5±0.3. Viscosity was in the range of 3210cp to 6000 cp.

Viscosity affected by concentration of Carbopol 934 and amount of water added. Spredability was in the range of 14.9±0.25 to 16.9±0.12gm/cm/sec&% drug content was in the range of 88.78±0.12 to 90.32±0.41 %.

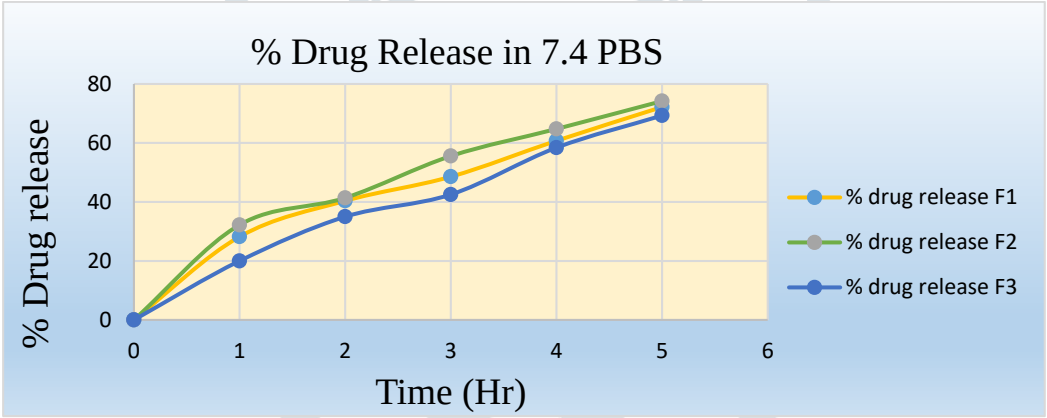

Fig.7: In-*vitro* drug release of Nanoparticles loaded gel

In vitro drug release was found to be in the range of 69.66±0.32 to 74.19±0.2% within 5 Hr. Hence G2 gel formulation was optimized based on good pH, viscosity, Spredability, % drug content (90.32±0.31 %) & % drug release (74.19±0.2 %). Irritancy score of optimized gel was zero i.e. gel was free from skin irritation.

Fig.8 Zone of inhibition of G2 gel loaded with nanoparticles.

The G2 gel formulation showed good inhibition zones at 75µg/l conc. i.e. 9 mm. So, its antibacterial activity against *P. acnes* was confirmed.

CONCLUSION

The current research demonstrates a simple, quick and cost effective approach for producing polymeric chitosan nanoparticles from *Cynodon dactylon*. Drug loaded chitosan nanoparticles were successfully prepared by cross linking with vanillin. The study concluded that when the concentration of polymer and cross-linker raises, PS and EE increases. It was observed that when conc. of Carbopol 934 raises viscosity increases & spredability decreases. So, the gel with 1% Carbopol 934 (G2) gel selected as optimized formulation based on good results compared with others. Hence, gel incorporated with chitosan vanillin cross-linked nanoparticles loaded with *C. dactylon* can be used as an appropriate formulation for the treat of *Acne vulgaris.*

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